

Claims

WHAT IS CLAIMED IS:

1. An isolated polynucleotide encoding a GlutRNA^{Gln} amidotransferase (AdT) protein.
2. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
 - (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide encoded by SEQ ID NO: 1; and
 - (b) a polynucleotide which is complementary to the polynucleotide of (a).
3. The polynucleotide of claim 2 wherein the polynucleotide is DNA.
4. The polynucleotide of claim 2 wherein the polynucleotide is RNA.
5. The polynucleotide of claim 3 comprising the nucleotides 103 to 3306 set forth in SEQ ID NO:1.
6. A vector comprising the DNA of claim 3.
7. A host cell comprising the vector of claim 6.
8. A process for producing a polypeptide comprising expressing from the host cell of claim 7 a polypeptide encoded by said DNA.
9. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of claim 6 such that the cell expresses the polypeptide encoded by the DNA contained in the vector.

10. A process for producing an amidotransferase polypeptide or amidotransferase fragment comprising culturing a host cell of claim 7 under conditions sufficient for the production of said polypeptide or fragment.
11. A polypeptide comprising an amino acid sequence which is at least 70% identical to a polypeptide encoded by nucleotides 103 to 3306 set forth in SEQ ID NO: 1.
12. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 11.
13. An antagonist which inhibits the activity of the polypeptide of claim 11.
14. A method for the treatment of an individual having need of amidotransferase comprising administering to the individual a therapeutically effective amount of the polypeptide of claim 11.
15. The method of claim 14 wherein said therapeutically effective amount of the polypeptide is administered by providing to the individual DNA encoding said polypeptide and expressing said polypeptide in vivo.
16. A method for the treatment of an individual having need to inhibit amidotransferase polypeptide comprising administering to the individual a therapeutically effective amount of the antagonist of claim 13.
17. A process for diagnosing a disease related to expression of the polypeptide of claim 11 comprising determining a nucleic acid sequence encoding said polypeptide.
18. A diagnostic process comprising analyzing for the presence of the polypeptide of claim 11 in a sample derived from a host.
19. A method for identifying compounds which bind to and inhibit an activity of a polypeptide of claim 11 comprising:

- (a) incubating a first sample of the polypeptide and its substrate;
- (b) measuring an uninhibited reactivity of the polypeptide from step (a);
- (c) incubating a first sample of the polypeptide and its substrate in the presence of a second sample comprising an inhibitor compound;
- (d) measuring an inhibited reactivity of the polypeptide from step (c); and
- (e) comparing the inhibited reactivity to the uninhibited reactivity of the polypeptide.

20. A method for identifying compounds which bind to and inhibit an activity of a polypeptide of claim 11 comprising:

- (a) contacting a cell expressing on the surface thereof a binding site for the polypeptide, said binding being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said binding site, with a compound to be screened under conditions to permit binding to the binding site; and
- (b) determining whether the compound binds to and activates or inhibits the binding by detecting the presence or absence of a signal generated from the interaction of the compound with the binding site.

21. A method for identifying inhibitor-resistant AdT mutants comprising:

- (a) incubating a first sample of wild-type AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;
- (b) measuring an unmutated reactivity of the AdT from step (a);
- (c) incubating a first sample of a mutated AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;
- (d) measuring a mutated reactivity of the mutated AdT from step (c); and,
- (e) comparing the mutated reactivity to the unmutated reactivity of the wild-type AdT.

22. A method for inducing an immunological response in a mammal which comprises inoculating the mammal with amidotransferase, or a fragment or variant thereof adequate to produce antibody to protect said animal from disease.

23. A method of inducing immunological response in a mammal which comprises, through gene therapy, delivering a gene encoding an amidotransferase fragment or a variant thereof for expressing amidotransferase, or a fragment or a variant thereof in vivo in order to induce an immunological response to produce antibody to protect said animal from disease.

24. An immunological composition comprising a DNA which codes for and expresses an amidotransferase polynucleotide or protein coded therefrom which, when introduced into a mammal, induces an immunological response in the mammal to a given amidotransferase polynucleotide or protein coded therefrom.

25. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

- (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising SEQ ID NO:4; and
- (b) a polynucleotide which is complementary to the polynucleotide of (a).

26. A vector comprising the polynucleotide of claim 25.

27. A host cell comprising the vector of claim 26.

28. A process for producing a polypeptide comprising expressing from the host cell of claim 27 a polypeptide encoded by said polynucleotide.

29. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid set forth in SEQ ID NO:4.

30. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 29.

31. An antagonist which inhibits the activity of the polypeptide of claim 29.

32. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

- (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising SEQ ID NO:6; and
- (b) a polynucleotide which is complementary to the polynucleotide of (a).

33. A vector comprising the polynucleotide of claim 32.

34. A host cell comprising the vector of claim 33.

35. A process for producing a polypeptide comprising expressing from the host cell of claim 34 a polypeptide encoded by said polynucleotide.

36. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid set forth in SEQ ID NO:6.

37. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 36.

38. An antagonist which inhibits the activity of the polypeptide of claim 36.

39. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

- (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising SEQ ID NO:8; and
- (b) a polynucleotide which is complementary to the polynucleotide of (a).

40. A vector comprising the polynucleotide of claim 39.
41. A host cell comprising the vector of claim 40.
42. A process for producing a polypeptide comprising expressing from the host cell of claim 41 a polypeptide encoded by said polynucleotide.
43. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid set forth in SEQ ID NO:8.
44. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 43.
45. An antagonist which inhibits the activity of the polypeptide of claim 43.
46. An isolated heterotrimeric protein comprising subunits A, B, and C, wherein:
- said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;
- said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;
- said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under

stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

47. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a protein of claim 46.

48. An isolated nucleic acid molecule that encodes a protein of claim 46.

49. A recombinant host that has been altered to contain a nucleic acid molecule of claim 48.

50. A method for producing an AdT protein comprising the step of culturing the host of claim 49 under conditions in which said introduced nucleic acid molecule is expressed.

51. A method to identify an agent that blocks translation, said method comprising the steps of:

- (a) contacting an agent with an AdT protein, or a subunit thereof; and,
 - (b) determining whether said agent binds to said AdT protein or said subunit;
- wherein said translation blocking agent is identified as being able to bind to said AdT protein, or said subunit.

52. The method of claim 51, wherein said AdT protein comprises a heterotrimeric protein consisting of an A, B and C subunit, wherein:

said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

53. The method of claim 52, wherein a single subunit of said AdT protein is used, and:

if subunit A is used, said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

if subunit B is used, said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

if subunit C is used, said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

54. The method of claim 51, wherein said agent is further tested for the ability to block the activity of said AdT protein.

55. The method of claim 54, wherein said AdT activity is tested in a cell free system.

56. The method of claim 54, wherein said AdT activity is tested in a cellular system.

57. A method to identify an agent that blocks translation, said method comprising the steps of:

- (a) contacting an agent with one or more of the subunits of an AdT protein;
- (b) incubating the three subunits of an AdT protein under conditions in which said subunits would associate to form an active AdT protein, wherein at least one of said subunits is from step (a); and
- (c) determining whether said agent blocks the association of said three subunits;

wherein said translation blocking agent is identified as being able to block the association of the subunits of said AdT protein.

58. The method of claim 57, wherein said AdT protein comprises a heterotrimeric protein consisting of an A, B and C subunit, wherein:

said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

59. The method of claim 57, wherein said agent is further tested for the ability to block the activity of said AdT protein.

60. The method of claim 59, wherein said AdT protein activity is tested in a cell free system.

61. The method of claim 59, wherein said AdT protein activity is tested in a cellular system.
62. A method to identify an agent that blocks translation, said method comprising the steps of:
- (a) contacting an agent with an AdT protein;
 - (b) determining whether said agent blocks the activity of said AdT protein.
63. The method of claim 62, wherein said AdT protein comprises a heterotrimeric protein consisting of an A, B and C subunit, wherein:
- said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;
 - said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;
 - said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.
64. The method of claim 63, wherein said AdT protein activity is tested in a cell free system.
65. The method of claim 63, wherein said AdT protein activity is tested in a cellular system.

66. A method to block translation of a protein within a cell, comprising the step of contacting said cell with an amount of an agent that binds to an AdT protein, or a subunit thereof, sufficient to block said translation.

67. The method of claim 66, wherein said agent binds to a subunit of said AdT and blocks the association of said subunits.

68. The method of claim 66, wherein said agent is used as an antibacterial agent.

69. The method of claim 66, wherein said agent is used as an antifungal agent.

70. The method of claim 66, wherein said agent is used as a herbicide.

71. An isolated polynucleotide that codes for a mutant AdT which confers resistance to an inhibitor of wild-type AdT.

72. A vector comprising the polynucleotide of claim 71.

73. A host cell comprising the vector of claim 72.

74. The host cell of claim 73 wherein the host cell comprises a plant cell.

75. A process for producing a polypeptide comprising expressing from the host cell of claim 73 a polypeptide encoded by said polynucleotide.

76. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of claim 73 such that the cell expresses the polypeptide encoded by the polynucleotide contained in the vector.

77. A process for producing a plant which comprises a gene for resistance to an AdT inhibitor, said process comprising regenerating a plant from the plant cell of claim 74.

78. A process of plant husbandry comprising:

- (a) planting a plant which comprises a gene for resistance to an AdT inhibitor;
- and
- (b) applying a herbicide which comprises an AdT inhibitor.